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# Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors

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Previous experiments in our laboratory suggested that ammonium toxicity could be mediated by the NMDA type of glutamate receptors. To assess this hypothesis we tested if MK-801, a specific antagonist of the NMDA receptor, is able to prevent ammonium toxicity. Mice and rats were injected i.p. with 12 and 7 mmol/kg of ammonium acetate, respectively. 73% of the mice and 70% of the rats died. However, when the animals were injected i.p. with 2 mg/kg of MK-801, 15 min before ammonium injection, only 5% of the mice and 15% of the rats died. The remarkable protection afforded by MK-801 indicates that ammonia toxicity is mediated by the NMDA receptor.

Ammonia toxicity; NMDA receptor; MK-801; Hyperammonemia

## 1. INTRODUCTION

Ammonia toxicity was first reported in the laboratory of Pavlov in 1893 [1]. Injection of large amounts of ammonia into animals results in their rapid death. However, in spite of much work, the mechanism of ammonia toxicity remains elusive. None of the hypotheses suggested has been fully supported by the experimental results. We have studied the effects of hyperammonemia on brain using a new animal model [2,3]. The results obtained indicate that ammonia markedly affects glutamatergic neurotransmission and suggest that ammonia toxicity could be mediated by activation of the NMDA type of glutamate receptors. To assess this hypothesis, we have tested if a highly specific antagonist of the NMDA receptor (MK-801) is able to prevent ammonia toxicity. It is shown that antagonists of the NMDA receptor markedly protect the animals against ammonia toxicity. This finding can be of great interest for the understanding of the mechanism of acute ammonia toxicity and the possible contribution of hyperammonemia to the pathogenesis of hepatic encephalopathy.

## 2. MATERIALS AND METHODS

Male Swiss albino mice weighing 25–30 g and male Wistar rats weighing 250–300 g were used. Mice and rats were injected intraperitoneally with 12 and 7 mmol/kg, respectively, of ammonium acetate. To assess if ammonia toxicity is mediated by the NMDA receptor we tested the possible protective effect of 2 antagonists, acting at different sites, of this receptor. MK-801 is a potent and selective antagonist which blocks the ion channel while 2-amino-5-phosphonopentanoic acid (AP-5) binds to the glutamate binding site. The experimental groups were injected i.p. with 2 mg/kg of MK-801 or 10–500 mg/kg of AP-5 15 min before injecting ammonium acetate.

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## 3. RESULTS AND DISCUSSION

Groups of mice were injected i.p. with 12 mmol/kg of ammonium acetate. As shown in Table 1, 73% of the mice died. However, when the mice were injected i.p. with 2 mg/Kg of MK-801 15 min before injection of ammonium acetate only 5% died. The same experiment was carried out with rats, except that rats were injected with 7 mmol/kg of ammonium acetate. 70% of the rats injected with ammonium acetate died compared to only 15% of those previously injected with MK-801. It should be noted that MK-801 did not prevent the somnolence, tachypnea and tonic convulsions produced by acute ammonia intoxication.

These results clearly indicate that MK-801 protects the animals from ammonia toxicity. MK-801 is a potent and selective antagonist of the NMDA type of glutamate receptors which bind to the ion channel of the receptor complex [4,5]. To further confirm that the protective effect of MK-801 is mediated by its action on the NMDA type of glutamate receptors, we assessed the effect of another specific antagonist, AP-5, which acts on a different site (the glutamate binding site) of the same receptor. AP-5 also markedly protects mice from ammonia toxicity; 80% protection was reached by injecting 500 mg of AP-5 per kg of body weight. As expected, large amounts of AP-5 were necessary to produce a protective effect because AP-5 acts at the glutamate binding site and must therefore compete with the endo-

Table 1

Protective effect of MK-801 against acute ammonia toxicity

| | MK-801 | Animals injected | Survivors | Survival (%) |
|---|---|---|---|---|
| Mice | no | 41 | 11 | 27 |
| | yes | 20 | 19 | 95 |
| Rats | no | 31 | 10 | 32 |
| | yes | 31 | 26 | 84 |

Mice and rats were injected i.p. with 12 and 7 mmol/kg, respectively, of ammonium acetate. The experimental groups were injected i.p. with 2 mg/kg of MK-801 15 min before injecting ammonium acetate.

genous glutamate present around the receptor. Antagonists of other unrelated receptors were ineffective, e.g. flumazenil, an antagonist of the GABA-benzodiazepine receptor, had no effect at all.

These results indicate that the toxicity of ammonia is mediated by the NMDA type of glutamate receptors. It has been clearly established that neurotoxicity of glutamate is mainly due to activation of the NMDA receptor which leads to a sustained increase in the intercellular level of $CA^{2+}$, which in turn, by mechanisms which are beginning to be clarified, leads to the death of the cell [6]. The same mechanism appears to be involved in neuronal damage and death induced by ischemia. Under ischemic conditions there is an induction of the release of glutamate which would activate the NMDA receptor and lead to neuronal damage and death. MK-801 also protects neurons from the damage produced by ischemia [7,8].

We have also found that the release of glutamate in brain is greatly increased in hyperammonemic rats. Our results suggest that injection of ammonium acetate induces a large relaese of glutamate which leads to the death of the animal by activation of the NMDA type of glutamate receptor. If this receptor is previously blocked by an antagonist (MK-801 or AP5) the toxic effect of ammonia is prevented.

Alcohols, by an unknown mechanism, protect mice against acute ammonium intoxication. [9]. It has been shown recently that alcohols inhibit NMDA-activated ion current on neurons [10,11]. Moreover, butanol which is a more potent inhibitor of the NMDA receptor than ethanol [10] has a greater protective effect against ammonium toxicity [9]. This suggests that the protective effect of alcohols could also be mediated by inhibition of the NMDA receptor and supports the finding that ammonia toxicity is mediated by this receptor. It should be noted that MK-801 has previously been used is humans as an anticonvulsant for epilepsy patients [12]. Therefore, in addition to the basic scientific understanding, the clinical implications of this work could also be of great interest.

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